



Short report

## Genetic composition of six miniSTR in a Brazilian Mulatto sample population

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### ABSTRACT

The present study characterizes the genetic variability of Mulatto population based on the polymorphism of six miniSTR autosomal loci, known as Non CODIS 01 and 02 (NCO1 and NCO2) and evaluate their applicability in forensic genetics. A sample of 102 unrelated Brazilian mulattoes were genotyped for miniSTR loci D1S1677, D2S441, D4S2364 (miniplex NCO2) and 45 individuals for D10S1248, D14S1434, D22S1045 (miniplex NCO1). No significant deviations from Hardy–Weinberg equilibrium expectations were detected. The combined power of discrimination (PD) and mean power of exclusion (PE) were 0.999996 and 0.98991, respectively. The results also support the effectiveness of the NCO1 and NCO2 miniplexes for human identification.

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## 1. Introduction

Brazil is a continental country that was colonized by the Portuguese. After the Portuguese, other ethnic-geographic populations immigrated to Brazil, mainly from Spain, Italy, Germany, and Japan. The National Survey Inquiries use to classify skin color in four categories: white, brown, black and yellow. The brown group corresponds to the so-called Mestizos, which are said to comprise three subsets of people in Portuguese language: "caboclos", a mixture of European and Amerindians; "mulatos", mixture of European- and African-descendants; and "cafusos", mixture of African-descendants and Amerindians. Among them, the mulattoes are far the main group in number. At the end of the XX Century, the Brazilian population was known to be composed as follows: 62.6% whites; 5.8% of blacks; 30.8% of mulattoes and 0.8% of other minorities.<sup>1</sup> The ethnic structure of the Brazilian southeast population, such as the one in Rio de Janeiro area, is considered complex and multicultural because it had received different migration waves. In order to increase the genetic information on admixture population, we have investigated the genetic data of the

six microsatellites, known as Non CODIS (NCO), to evaluate their applicability in forensic genetics.

Blood samples were collected from a total of 102 unrelated Brazilian mulattoes, living in Rio de Janeiro.

## 2. Materials and methods

### 2.1. Extraction

Genomic DNA was prepared by salting-out extraction procedure.<sup>2</sup>

### 2.2. PCR

The NCO1 (D10S1248, D14S1434, D22S1045) and NCO2 (D1S1677, D2S441, D4S2364) miniplexes were amplified following the parameter outlined elsewhere.<sup>3,4</sup> Amplified products were electrophoresed on a 3130xl Genetic Analyzer (Applied Biosystems) with a 36 cm capillary array, POP-7 polymer and 10 s at 3000 V injections, and separated for 15 min at 15 kV at 60 °C. GeneScan™-500 LIZ™ was used as internal size standard.

### 2.3. Typing

Allelic designation with the corrected nomenclature<sup>5</sup> was determined using GeneMapper® 3.2 software (Applied Biosystems),

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**Table 1**

Allelic frequencies, statistical and forensic measures for six miniSTR loci from Rio de Janeiro Mulatto DNA samples.

Alleles	Locus					
	D1S1677	D2S441	D4S2364	D10S1248	D14S1434	D22S1045
8	—	—	0.162	—	—	—
9	—	—	0.627	—	—	—
10	—	0.181	0.211	0.011	0.144	—
11	0.005	0.319	—	0.033	0.067	0.122
11.3	—	0.064	—	—	—	—
12	0.123	0.157	—	0.111	0.089	0.022
13	0.221	0.020	—	0.244	0.233	0.044
14	0.294	0.235	—	0.256	0.422	0.389
15	0.284	0.025	—	0.178	0.044	0.322
16	0.049	—	—	0.133	—	0.100
17	0.020	—	—	0.033	—	—
18	0.005	—	—	—	—	—
N	204	204	204	90	90	90
MP	0.099	0.083	0.279	0.097	0.120	0.134
PD	0.901	0.917	0.721	0.903	0.880	0.866
PIC	0.73	0.75	0.48	0.78	0.70	0.67
PE	0.606	0.485	0.163	0.818	0.411	0.446
PI	2.55	1.89	0.94	5.63	1.61	1.73
He	0.770	0.784	0.538	0.820	0.740	0.726
Ho	0.804	0.735	0.471	0.911	0.689	0.711
p	0.952	0.459	0.094	0.085	0.2125	0.104
Fis	−0.045	0.063	0.126	−0.113	0.070	0.022

N, numbers of chromosomes. MP, Match Probability. PD, Power of Discrimination. PIC, Polymorphic Information Content. PE, Power of Exclusion. PI, Typical Paternity Index. He, Expected Heterozygotes. Ho, Observed Heterozygotes. Hardy–Weinberg Equilibrium *p* value. Fis, Fixation index.

calibrated with standard DNA cell lines, such as K562, 9947A, 9948 and 007 ([www.cstl.nist.gov/div831/strbase/miniSTR/miniSTR\\_NC\\_loci\\_types.htm](http://www.cstl.nist.gov/div831/strbase/miniSTR/miniSTR_NC_loci_types.htm)).

#### 2.4. Quality Control

All methods and procedures conducted during this study followed the established quality assurance measures developed by the laboratory and certified under ISO 17025.

#### 2.5. Statistical analysis

Statistical parameters were obtained using PowerStat version 1.2 (Promega Corp.). Hardy–Weinberg Exact Test and Linkage Disequilibrium were performed using the GDA Package.<sup>6</sup> Genetic distances (Fst, Table 1) for the Brazilian Mulattoes and population samples from Portugal,<sup>7</sup> Argentina,<sup>8</sup> Italy,<sup>9</sup> Japan,<sup>10</sup> China<sup>11</sup> and US Afro-Americans<sup>4</sup> were calculated from allelic frequencies using Power Marker software.<sup>12</sup> TreeView software was used for graphic representation.<sup>13</sup> The software SPSS v.14 was used to produce the multidimensional scale plot.

**Table 2**

Fst matrix of population groups: Mulatto, Portuguese, Argentinian, Italian, Japanese, Chinese, and Afro-American. Above diagonal, *p* values. In bold, significant Fst values (*p* < 0.05).

	Mulatto*	Portuguese <sup>7</sup>	Argentinian <sup>8</sup>	Italian <sup>9</sup>	Japanese <sup>10</sup>	Chinese <sup>11</sup>	African–American <sup>4</sup>
Mulatto	—	0.1733	0.7033	0.1015	<b>0.0000</b>	<b>0.0000</b>	<b>0.0005</b>
Portuguese	0.0101	—	0.9956	0.2378	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>
Argentinian	0.0092	0.0059	—	0.0563	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>
Italian	0.0115	0.0104	0.0166	—	<b>0.0000</b>	<b>0.0000</b>	<b>0.0002</b>
Japanese	0.0931	0.1030	0.0954	0.0937	—	0.0991	<b>0.0000</b>
Chinese	0.0666	0.0809	0.0748	0.0767	0.0224	—	<b>0.0000</b>
African–American	0.0171	0.0328	0.0372	0.0205	0.0989	0.0674	—

Fst matrix of population groups \*this paper. Above diagonal, *p* values. In bold, significant Fst values (*p* < 0.05).

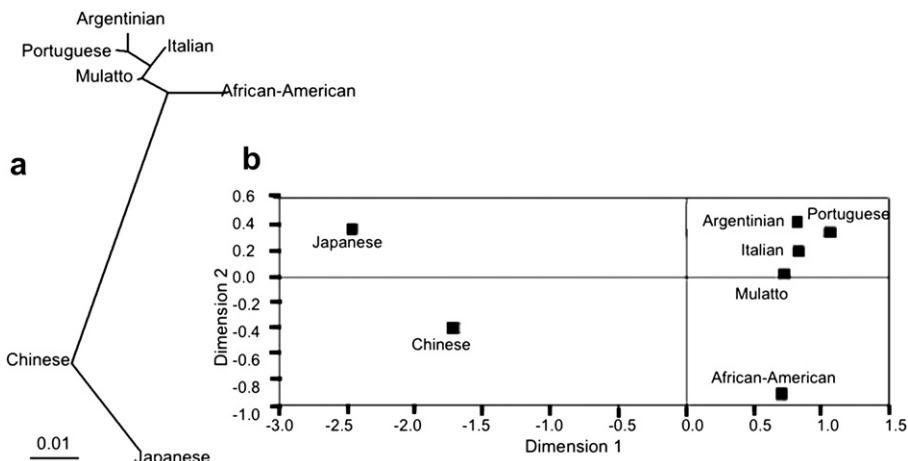
### 3. Results

#### 3.1. Analysis of data

Allele frequency distribution, statistical and forensic parameters, regarding the six autosomal miniSTR loci, were determined in 45–102 individuals, and the results are shown in Table 1. Microsatellite data reveals that the gene diversity values range from 0.538 for D4S2364 to 0.82 for D10S1248. The average gene diversity, or expected heterozygosity, is 0.729, which is similar to other populations.<sup>4,7–11</sup> All markers have shown no significant deviations from Hardy–Weinberg expectations based on the Fisher's Exact Test after 10,000 shuffles considering *P* < 0.05. Linkage disequilibrium between all pair of loci was evaluated with all combinations returning a probability greater than 0.05, indicating independence of the loci (data not shown). Linkage disequilibrium was also assessed between the NC01 and 02 miniplex loci and the Promega PowerPlex 16 loci with no deviations from independence observed (data not shown). The estimation of the fixation index, or inbreeding coefficient, was performed by the calculation of Fis. The level of fixation index for each marker ranged from −0.113 to 0.126 for D10S1248 and D4S2364, respectively. For all loci the total value of Fis was 0.014 indicating no population sub-structure.

#### 3.2. Other remarks

Brazilian population is known to be heterogeneous regarding the ethnic composition and geographic distribution.<sup>1</sup> The relationship between Rio de Janeiro mulattoes and other populations were investigated and the Fst genetic distances (Table 2). Genetic distance were significant different (*p* < 0.05) in three out of six pairwise comparison (Japanese,<sup>10</sup> Chinese,<sup>11</sup> African–American<sup>4</sup>). The data have shown close-proximity between Rio de Janeiro Mulattoes and European population samples such as Portuguese<sup>7</sup> (*p* = 0.1733), Italian<sup>9</sup> (*p* = 0.1015) and the European-descendant population sample such as Argentinian<sup>8</sup> (*p* = 0.7033). Although the Mulattoes may have African background, the Fst distribution of mini STRs suggests that the Europeans are the major genetic contributor. Even so the NJ tree (Fig. 1a) imposes a bifurcating model onto a distance matrix, which may be inadequate for closely related populations, we performed a multidimensional scale analysis (MDS) (Fig. 1b), which resembles the same tree topology. In our previous STR Brazilian data,<sup>14</sup> we have found no significant distances, but this work addresses the comparison of European and African derived population from US, not from Brazil. As pointed before, Brazilian population has a higher degree of admixture than American population, leading the present results. In addition, the use of the six miniSTR loci is suitable for forensic investigations with a combined power of discrimination (PD) for the NCO 1 & 2 panels of 0.999996.



**Fig. 1.** (a) Neighbor-joining tree using  $F_{ST}$  as a measure of genetic distance and (b) Multidimensional analysis using  $F_{ST}$  matrix among Rio de Janeiro Mulatto, Portuguese, Argentinian, Italian, Japanese, Chinese and US African–American groups (coefficient of stress: 0.025). Reference population as in Table 2.

#### 4. Access to data

Through e-mail to the corresponding author.

#### Conflict of interest

None declared.

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#### Ethical approval

Samples were made anonymous after the collection procedure approved by the Research Ethics Committee from Instituto de Pesquisas e Perícias em Genética Forense da Polícia Civil do Estado do Rio de Janeiro, Brazil, CEP/IMLAP/DPTC Number 006/2006.

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